ELECTROPROCESSED COLLAGEN

This application is a continuation-in-part of U.S. Patent Application Serial No. 09/512,081, filed February 24, 2000, which is a continuation-in-part of U.S. Patent Application Serial No. 09/386,273, filed August 31, 1999. The '081 application also claims priority, in part, from U.S. Provisional Application Serial No. 60/121,628, filed on February 25, 1999.

This invention relates to the product of electroprocessing collagen. Because collagen is a commonly-found natural polymer, there are many varied uses for a matrix of electroprocessed collagen fibers and/or droplets.

Background of the Invention

Many different types of synthetic polymers are used to form structures or platforms in biomedical applications. Bandages and patches are formed of woven polymer fibers. Prosthetics can be made of different polymers and combinations of polymers. Vascular sleeves, for instance can be made of woven fibers. Several problems with using man-made polymers for biomedical applications include difficulty in mimicking natural tissue and likelihood of rejection by a body's natural defense mechanisms.

In order to overcome problems with using synthetic polymers, a natural polymer such as collagen can be used. Collagen is conventionally used in biomedical applications as a gel or foam. (E.g., U.S. Patent No. 5,891,588). There are limitations to this use of collagen as a result of the inherently unoriented nature of a collagen gel or foam. In order to obtain orientation and more closely mimic biological conditions, collagen is typically processed, if at all, by extrusion

techniques. These techniques are limited by diameter or thickness of fibers or sheets available from the equipment used.

In another field, a common, naturally-occurring product that incorporates collagen is leather. Artifical leather products made of, for instance, vinyl are known, but they are not as desirable as natural leather itself. Leather is a well known product that has been used for thousands of years and for a large range of applications. Leather is a material for making different clothing and fashion accessory products such as coats, gloves and purses, among other things. Leather is used in the manufacture of shoes and boots. Leather is used to form different upholstery products in the home and, for instance, in automobiles. Leather is also used as an industrial product with numerous applications.

There are limitations inherent in the use of leather as a raw material. Leather has naturally occurring imperfections, which means that it is difficult to obtain a consistent product in consistent supply. It is necessary to adapt any specific application for leather product to available leather products instead of adapting the leather to the application. For instance, seams are needed in the formation of complex shaped leather goods. Also, it is virtually impossible to repair tears or holes in leather products.

In addition to biomedical platforms and leather, collagen is a common food casing. For instance, meat products can include an outside casing that incorporates collagen. A well-known example is sausage where a casing of animal intestine (collagen) is filled with seasonings, meat and other ingredients. Drawbacks with conventional meat casing technologies include a limited range of diameters of casings that are available and limited consistency with the quality of those casings.

In still another separate field, the textile industry has used for many years the process of electrospinning polymer fibers to make fabrics and nonwoven products. The polymers employed are typically polyester and other synthetic polymers. Traditionally, sheets of electrospun polymer fabric are formed and then processed (cut and sewn, for instance) just like any other sheets of woven or knit fabric.

Summary of the Invention

Accordingly, it is an object of the present invention to overcome the foregoing limitations and drawbacks by using collagen as the polymer in the process of electroprocessing. By electroprocessing collagen, a matrix can be formed for use in multiple fields of use including biomedical applications, food casing applications, manufactured leather applications, and footwear and clothing applications, among others.

In one embodiment, the invention is the product of the process of electroprocessing collagen. This processing may comprise electrospinning collagen fibers, or it may comprise electrospraying collagen droplets. Further, the collagen may be synthetically manufactured collagen or collagen produced by genetic engineering or a subset of a collagen molecule such as a specific sequence of amino acids contained within a larger collagen protein molecule.

A method for making a matrix of collagen includes providing a substrate and a reservoir of solution including collagen wherein the reservoir has an orifice that allows the solution to leave the reservoir. Then, either the substrate or the solution is electrically charged and the other is grounded. The collagen is then streamed from the reservoir and through the orifice onto the substrate to form a matrix. The step of streaming collagen may form a matrix of collagen fibers. It may, alternatively, form a matrix of collagen droplets. Further, the substrate may define a

preselected shape. The collagen matrix can be formed in the presence of cross-linking agents or may be treated with a cross-linking agents after streaming.

A further method for making a matrix of collagen includes providing a substrate, a target, and a reservoir solution including collagen wherein the reservoir has an orifice that allows the solution to leave the reservoir. Then, either the target or the solution is electrically charged and the other is grounded. The substrate is disposed between the orifice and the target. The collagen is then streamed from the reservoir and through the orifice onto the substrate to form a matrix. The step of streaming collagen may form a matrix of collagen fibers. It may, alternatively, form a matrix of collagen droplets. Further, the substrate may define a preselected shape. The collagen matrix can be formed in the presence of cross linking agents or may be treated with cross linking agents after streaming.

In still a further embodiment, there is a food casing comprising a matrix of electroprocessed collagen. The collagen may be electrospun collagen fibers. Alternatively, it may be electrosprayed collagen droplets. The collagen matrix may be cross linked or otherwise processed to alter its structural or chemical properties.

In a still further embodiment, manufactured leather comprises a matrix of electroprocessed collagen. The collagen may include electrospun collagen fibers. Alternatively, the matrix may include electrosprayed collagen droplets. Also, the collagen may be cross linked or otherwise processed to alter its structural or chemical properties.

Brief Description of the Drawings

Figures 1 and 2 are scanning electron micrographs of different magnification of the matrix of electroprocessed collagen as described in Example 1.

Figure 3 is a transmission electron micrograph of the matrix of electroprocessed collagen as described in Example 1.

Figures 4 and 5 are scanning electron micrographs of different magnification of a matrix of electroprocessed collagen/elastin as described in Example 2.

Figure 6 is a scanning electron micrograph of the matrix of electroprocessed collagen and elastin as described in Example 3.

Figures 7 through 9 are scanning electron micrographs of varying magnification of electroprocessed collagen to form leather as described in Example 4.

Figures 10 through 12 are scanning electron micrographs of a matrix of collagen that has been electroprocessed and treated with a cross linking agent to form leather as described in Example 4.

Detailed Description of Preferred Embodiments

This description includes different examples of electroprocessing techniques. There are other examples contained in U.S. Patent Applications Serial Nos. 09/512,081, 09/386,273 and U.S. Provisional Application Serial No. 60/121,628. Those applications are incorporated herein by reference as if set forth in their entirety.

The term "electroprocessing" shall be used broadly to cover the methods of electrospinning of fibers, electrospraying (electroaerosoling) of droplets, combinations of electrospinning and electroaerosoling, and any other method where a polymer including collagen

is streamed across an electric field. The solution being streamed may be charged and directed to a grounded substrate. Similarly, the solution may be streamed from a grounded reservoir in the direction of a charged substrate. The term "electroprocessing", therefore, is not limited to the specific examples set forth herein.

Throughout this application, the term "solution" is used to describe the liquid in the reservoir of the electroprocessing method. This could imply that the collagen polymer is fully dissolved in the liquid. In this application, the term "solution" also refers to suspensions when the collagen is not soluble (or only partially soluble) in the liquid used in a given process. This broad definition is appropriate in view of the large number of solvents or other liquids that may be used in the many variations of electroprocessing.

Also, the term "collagen" is used throughout in its broadest definition. There are multiple types of collagen that are naturally-occurring as well as types that are being synthetically manufactured or produced by genetic engineering. Other types may be found or synthesized in the future, or specific subsets of various collagen molecules may be isolated with unique effects and may be used to make products like those described herein. All of these types and subsets are encompassed in the use of the term "collagen" herein. Also, as evidenced by some of the examples, the collagen may be mixed with other polymers during electroprocessing to obtain specifically desirable properties for given end use of the matrix.

There are many different applications for electroprocessed collagen. The versatility is enabled by the variability of the process itself. Generally speaking, there is variability with the equipment used, the solution that is streamed in the process, and various post-process treatments.

In the most fundamental sense, the electroprocessing apparatus includes a streaming mechanism and a target substrate. The streaming mechanism will include a reservoir or

reservoirs to hold the solution that is to be streamed in the process. The reservoir or reservoirs have at least one orifice or nozzle to allow the streaming of the solution from the reservoirs. There may be a single nozzle or there may be multiple nozzles in a given electroprocessing apparatus. If there are multiple nozzles, they may be attached to one or more reservoirs containing the same or different solutions. Similarly, there may be a single nozzle that is connected to multiple reservoirs containing the same or different solutions. Also, the size of the nozzle may be varied to provide for increased or decreased flow of the solution out of the reservoir through the nozzle. A pump used in connection with the reservoir may be used to control the flow of solution streaming from the reservoir through the nozzle or nozzles. The pump may be programmed to increase or decrease the flow at different points during an electroprocessing run.

The target substrate may also be used as a variable feature in the electroprocessing of polymers such as collagen. Specifically, the target may be the actual substrate onto which the polymers including collagen are deposited. Alternatively, a substrate may be disposed between the target and the nozzle. For instance, a petri dish can be disposed between a nozzle and a target, and a matrix can be formed in the dish to study cell growth in 3-D by having a scaffold in the bottom of the dish. Other variations include non-stick surfaces between the nozzle and target. The target may also be specifically charged (grounded) along a preselected pattern so that the polymer streamed from the orifice is directed into specific directions. Ideally, the electric field is controlled by a program to create a matrix having a desired geometry. The target and the nozzle or nozzles may be engineered to be movable with respect to each other thereby allowing additional control over the geometry of the matrix to be formed. It is envisioned that the entire process will be controlled by a microprocessor that is programmed with the specific parameters

to obtain a specific, preselected electroprocessed matrix of collagen and, if desired, other polymers.

Also, as noted in the specific examples that follow, the nozzle or orifice that allows streaming of solution from the reservoir is shown to be charged and the target is shown to be grounded. Those of skill in the electroprocessing arts will recognize that the nozzle and solution may be grounded and the target may be electrically charged. In any event, it is the creation of the electric field and the effect of the electric field on the streamed collagen that helps create the unique collagen matrix.

In addition to the multiple equipments and variations and modifications that can be made to obtain desired results, similarly the solution can be varied to obtain different results. For instance, the solvent or liquid in which the collagen is dissolved or suspended may be varied. The collagen can be mixed with other polymers to obtained desired end results. In still a further variation, when multiple reservoirs are used, the ingredients in those reservoirs may be electrosprayed separately or joined at the nozzle so that the ingredients in the various reservoirs may react with each other simultaneously with the streaming of the solution into the electric field. Also, when multiple reservoirs are used, the different ingredients in different reservoirs may be phased in over time in the processing period. The collagen may be directly altered, for example, by altering the carbohydrate profile of the molecule. Also, other materials may be attached to the collagen before, during or after electroprocessing. Further, the temperature and other physical properties of the process can be modified to obtain different results.

Finally, there are many types of post-process treatments that may be used to modify and adjust the matrix that is the result of the electroprocessing procedure. For instance, a matrix of electroprocessed collagen may be treated with a cross linking agent, including chemical and UV-

light based cross-linking agents. Also, the matrix may be treated with variations in temperature. Still further chemical variations may be envisioned by those desiring specific end properties of a matrix.

Having considered some of the variations in the process that are available, it is clear that there are multiple different applications that would benefit from the electroprocessing of collagen. The different fields of use of this process and the product thereof include biomedical applications, food casing applications, manufactured leather applications, and clothing and footwear applications. Of course, these are not the only applications possible for an electroprocessed matrix of collagen. Examples of potential applications in these fields of use will be discussed in the following.

Biomedical Applications

There are at least two major biomedical applications for electroprocessed collagen matrices. In general terms, there is use of a matrix in the formation of an extracellular matrix and in the formation of a drug delivery platform.

Specific applications and variations where electroprocessed collagen is used as a drug delivery platform are described in provisional application entitled entitled Electroprocessing In Drug Delivery And Cell Encapsulation, Serial No. ______________, filed October 18, 2000, which is incorporated herein by reference as if set for in its entirety.

Some of the variations and potential applications for use of an electroprocessed matrix of collagen as an extracellular matrix are discussed in detail in prior filed pending applications, Serial Nos. 09/386,273, 09/512,081 and 60/121,628. Those applications are incorporated herein by reference as if set forth in their entirety.

Electrospun collagen has a repeating, banded pattern when it is examined by electron microscopy. This banded pattern is typical of collagen fibrils produced by natural processes (i.e. banded pattern is observed in collagen when it is produced by cells). Most collagen fibrils produced in vitro lack this banded appearance. This banded pattern is an important attribute because it allows cells to have access to active sites within the collagen molecule that promote or regulate specific activities. For example, the AP-15 site, a 15 amino acid sequence within the collagen molecule, promotes bone cells (for example osetoblasts) to produce and secrete hydroxyapatite, a critical component of bone. In many of the types of collagen fibrils that are produced in vitro, the AP-15 site is cryptic and hidden. When osteoblasts are placed onto a collagen matrix with a cryptic AP-15 site, they fail to express bone specific components. By modulating the electrospinning system, different binding sites can be selectively hidden or exposed to control cell behavior. In addition, collagen may be enriched with specific sequences during, after or before the electrospinning process. Furthermore, specific subsets of collagen polymers, such as the AP-15 site can be produced and selectively electrospun so that the entire matrix is composed of just polymers of the AP-15 binding sites. Other specific sites and sequences within the collagen molecules may be manipulated and processed in a similar fashion-for example the RGD binding sites of the integrin molecule. Increasing the amount of RGD binding sites within a matrix might be used to increase the efficiency or strength of attachment of cells to a collagen matrix.

In addition to the extracellular matrix (tissue scaffold) applications described in the earlier-filed applications, the following are further applications for use of electroprocessed collagen as an extracellular matrix.

1. Vascular Valves - Natural material or collagen derived from genetic engineering or an

entirely synthetic source, with or without cells could be used in this application. A valve, for example a cardiac, aortic, or venus valve, could be assembled in vitro most likely with a bioreactor for leaflet preconditioning. The advantage of utilizing electroprocessing is that it affords one the ability to mimic the architecture of the collagen in a native leaflet. Many variations could be accounted for in the production of such a valve. One variation is a design where a ring is formed around the edge of the valve that is thickened like the natural valve. This ring provides a means of attachment and structural support.

2. <u>Tendon/Muscle Repair</u> – A matrix is formed in the shape of a sleeve that is hollow and shaped like a funnel. At the base of the funnel, the solid tendon will project. The sleeve part can fully or partially envelop the muscle belly. This would allow a firm attachment or a not so firm attachment that could be gradually loaded as the muscle cells grow into the new tendon. This method could be utilized in vitro but also in vivo for tissue repair/regeneration. Increasing the number of RGD binding sites within an electrospun matrix of collagen used in a tendon might be used to increase the adhesion of muscle cells to the matrix.

3. <u>Tendon or Ligament</u> - An entire ligament can be spun with the slight twist created by such means as a rotating nozzle or air vortex. The collagen can be autologous in nature (this holds true for the all possible utilizations of electrospun collagen). One possibility for creating a ligament would be to harvest the damaged ligament, grind it up in a crude mixture and then electroprocess the new ligament. This would take place on the hours time scale so that one may harvest one day, spin, and reimplant the next day. It might be possible to electroprocess on sight in situ.

4. <u>Vascular Scaffold or Support</u> - An additional product is not an artery but an aneurysmal sleeve. This is done by making a collagen sleeve with or without additional reinforcement such

as a suture or another thick fiber. One could spin a tube, cut the aneurysm (in most cases one may not wish to cut the aneurysm but simply wrap it or wrap one which has torn), and then slip the vascular support over the transected/torn artery. Once in place, the surgeon could then stitch the support directly to the arterial wall. The sleeve covers the aneurysm sight and acts like a sock surrounding it to reinforce its structure.

5. Wound Care – A dressing can be applied topically. This could be a simple bandage to a complex wound packing for a diabetic type ulcer or bed sore. Again, note that drug impregnation (antibiotics, hormones, angiogenic factors etc.) during or after electrospinning of the fibrous mats is a strong component for wound care products. Gene delivery is another possible application by incorporating vectors or naked genetic material in the spinning process to be incorporated by the cellular components during consumption and reorganization of the electrospun matrix. Also, one could incorporate oligoneculotides into the matrix. Antisense and sense oligoneculotides or even full length gene sequences could be added to provide transient control of gene expression. As the matrix is altered, the oligonucleotides would be released and become available to the cells. Another possible addition would be protease inhibitors. Also, one could incorporate peptides that physically repel or attract cells depending on a desired response. Materials such as chondroitin sulfate can be added to the matrix during after or before electrospinning to modify its structure. Chondroitin sulfate is used to stabilize collagen against heat denaturation and is a natural cross-linking agent.

6. Cartilage - Again, this could be autologous. In this case, hyaluronic acid, either natural or synthetic versions that are or are not subject to breakdown can be added to the collagen prior, during or after electroprocessing to promote hydration of the engineered tissue. Proteoglycons

can be added to more closely mimic cartilage. One could also add cells in vitro and/or in vivo depending on final designs.

7. Hernia Patch - Basically this is the same concept as the aneurysm sleeve except to repair hernias. Instead of a sleeve, the hernia patch would consist of a sheet of electrospun collagen. Additional natural materials such as elastin or synthetic materials could be added prior to, during or after electrospinining. This product would replace the Teflon patches currently used.

8. Nerve Repair - Seamless tubes of collagen, with or without biologically active molecules such as nerve growth factor or other materials such as various drugs can be used to facilitate repair of damaged or severed nerves.

9. Bone - Collagen mats or tubes can be seeded with osteoblasts with and without biologically active molecules such as bone morphogenic protein to generate bone of various shapes and sizes used, for example, for maxillofacial repair. As noted specific subsets of collagen molecules such as the AP-15 site can be used to alter the biological activity of an electrospun matrix. A matrix composed entirely of the AP-15 sequence could be electrospun or a matrix partially enriched with the AP-15 site or other sites could be fabricated.

10. Suture Material - Threads or cables of collagen could be produced by electrospinning for use as sutures. An electrospun suture based on collagen would be biocompatible as well as bioresorable. As with other materials fabricated from collagen the material and or chemical properties of the electrospun suture could be controlled prior, during, or after the fabrication process. Notably the suture material could be designed as a drug delivery system to suppress inflammation, promote healing, etc. Scaling the suture to larger sizes, i.e. producing a thicker thread rather than a fine thread, would allow filaments to be used in other applications. For

example, given sufficient thickness or strength, a synthetic catgut for stringing tennis rackets could be fabricated from collagen.

11. Bioengineering Platform - An additional application of the invention is as a platform for the fabrication of tissue using an electrospun matrix as a solid support. The fabrication platform is composed of an electrospun matrix of collagen, or other microfibers or blends of material. The overall three-dimensional geometric shape of the platform is determined by the ultimate design and type of tissue to be bioengineered.

Several permutations of the design are possible. For example, to fabricate a solid three-dimensional "plug" of tissue, a fabrication platform is electrospun on a mandrel. The mandrel may be cylindrical in shape, a flattened oval shape, a rectangular envelope shape (like a mailing envelope) or any other desired shape. The bioengineering platform was electrospun on a mandrel with the desired shape and allowed to "dry". The electrospun matrix was removed from the mandrel. For a cyclindrical-shaped bioengineering platform or any other shape of construct in which an enclosed area is desired, a suture, glue, staple or heat seal or some other method may be used to seal one end of the bioengineering platform. This results in a hollow platform that is closed on one end and open on the other. The electrospun platform can now be filled with cells or other materials, or cells or other materials may be placed on the outer surface of the construct. For example, a mixture of collagen or other materials and cardiac or skeletal muscle cells or any other desired cell type may be placed within the platform. The free and open end of the envelope that was used to fill the construct with material can be sutured, glued or heat sealed shut to produce an enclosed bioengineering platform. The entire construct is then placed into a bioreactor for cell culture or directly placed in situ for further development. With modifications, a bioengineering platform composed of a solid, rather than a hollow, format can be electrospun.

As before specific subsets of the collagen molecules or fiber blends may be used to form the matrix or supplement the material, physical, structural or chemical properties of the bioengineering platform. For example, the AP-15 site sequence might be added to a solution of collagen during electroprocessing, attached to collagen prior to processing, after electrospinning, streamed from a separate source, or even streamed alone to form a matrix.

Variations to this tissue engineering process includes the following:

- Add endothelial cells to the core or outside surface of the bioengineering platform during seeding to pre-form a capillary network.

- Form a cylinder and seed the outer surface with smooth muscle cells to form an arterial construct. The inner surface can then be seeded separately with endothelial cells to form an endothelial lining.

- Mix/add cells during the electroprocessing process to directly trap or surround cells within the matrix as it is in the process of forming. This may be accomplished by adding cells from a separate nozzel(s) or other sources if the materials to be electroprocessed must be placed in an organic solvent or solvents that will not support life for some other reason (for example a solvent with high or low pH or a solvent with high or low salt content).

- Cell polarity can be regulated by controlling the orientation of the fibers on the mandrel during or after electrospinning. For aligned fibers, this can be accomplished by electrospinning onto a target mandrel that is spinning. The fibers will be wrapped around the mandrel in the

direction of rotation. Electrospinning onto a static, non-spinning mandrel will produce a more random fibrillar matrix.

- Polarity may also be controlled by first electroprocessing an aligned matrix onto a spinning mandrel. The matrix is then cut from the mandrel, rotated 90 degrees (or any other degree of rotation) and placed back onto a mandrel. A second layer of material is then electrospun onto the first layer. This method will produce an inner layer of fibers that are aligned along the long axis of rotation.

- Cell polarity can be regulated by placing the bioengineering platform within a stretching device installed in the bioreactor. By gradually applying strain across the construct over time the cells within the platform will spread in parallel with the applied force.

- Mix cells in suspension with the fabrication platform as it is within the bioreactor to coat the exterior and or interior surfaces. For example, add tendon fibroblasts to the exterior of a skeletal muscle "plug" to establish the exterior architecture.

- Use the electrospun bioengineering platform as a differentiation platform for the manipulation of stem cells. The porosity and chemical composition of the electrospun matrix can be controlled prior, during, and after fabrication. This will allow an investigator to create the type of microenvironment that is believed to be critical for controlling the differentiation process in stem cells.

- Electrospin conductive materials into the matrix. In this way the construct can be electrically stimulated to promote neural ingrowth or contraction of engineered muscle. Incorporating conductive materials into an electrospun matrix also could be used as means to further alter the properties of a matrix following fabrication. For example, applying an electrical field across an existing matrix might be used to alter the shape, porosity or density of the matrix. The stability of the matrix (resistance to breakdown) might be altered –either increased or decreased, by applying an electric field across the filaments.

- Electrospin magnetically active materials into the matrix. In this way, the construct could be induced to alter shape or position by applying a magnetic field across the bioengineering platform. For example, applying a magnetic field across an existing matrix might be used to alter the shape, porosity or density of the matrix. The stability of the matrix (resistance to breakdown) might be altered –either increased or decreased by applying a magnetic field across the filaments.

- Place the engineered tissue within the omentum for vascularization.

- Place the engineered tissue or electrospun matrix directly in situ.

There are advantages to this tissue engineering process. It provides a solid support that can be used to grow tissue at very high density. It controls and establishes a local microenvironment with the construct. By controlling material properties of the matrix, one can control the buoyant nature of the construct, the porosity and the stability of the matrix (i.e. it can be designed to be very stable or to degrade over a relatively short period of time). It provides a platform for cell culture or tissue bioengineering that is unique and amenable for use in a bioreactor environment.

And it provides a platform for cell culture and bioengineering of many different types of tissue that is large and can be manipulated manually.

Example 1

The collagen used was Type I (calf skin, Sigma Chemical Co.). The collagen was suspended in 1,1,1,3,3,3- hexafluoro-2-propanol (HFP) at a concentration of 0.1181grams in 3 ml HFP. Once in solution or suspension (solution a milky color), the solution was loaded into a 1 ml syringe plunger. A 15-gauge luer stub adapter was then placed on the syringe to act as the electrospinning nozzle and charging point for the contained collagen solution. The filled syringe was placed in the KD Scientific's syringe pump set to dispense the solution at rate of 18 ml/hr utilizing a Becton Dickinson 1.0-ml syringe plunger. The positive lead from the high voltage supply was attached to the luer stub adapter metal portion. The syringe pump was turned on and the high voltage supply turned on and set at 20 kV. The grounded target was a 303 stainless steel mandrel (0.6 cm W x 0.05 cm H x 4 cm L) placed approximately 6 inches from the tip of the adapter. The mandrel was rotated at approximately 500 rpm during the spinning process. In the experiment, 1 ml of the collagen solution was electrospun to form a nice, white mat on the grounded mandrel. After electrospinning, the collagen mat was removed from the mandrel and processed for scanning electron microscopy evaluation. The results of this fibrous mat production can be seen in Figures 1 and 2. (Magnification 1000X and 4300X respectively). The mat produced was approximately 200 microns thick.

Transmission electron microscopy (TEM) evaluation was done and is shown in Figure 3. This cross-sectional micrograph illustrates the approximate 100 nm collagen fiber diameter and the typical 64 nm banding indicative of native collagen polymerization.

Example 2

The methods for this example are the same as Example 1 except for the electrospun solution. In this case, the spinning solution consisted of 0.1155 grams collagen, 0.1234 grams of elastin from ligamentum nuchae (Fluka), and 5 ml HFP. In this experiment, 2 ml of the suspension was spun to form the mat. The results of this experiment are shown in Figures 4 and 5. (Magnification 1800 and 6500 respectively). The mat produced was approximately 50 microns thick.

In this example, elastin was incorporated. For additional variations, other polymers, peptides, growth factors, biochemicals may be added during or after the spinning process to the collagen mats produced.

Example 3

The methods for this example are the same as Example 1 except for the electrospun solution. In this example, the solution electrospun was composed of 0.067 grams of type I collagen, 0.067 grams of type III collagen, 0.017 grams of elastin from ligamentum nuchae, and 2 ml HFP (44% Type I, 44% Type III, and 12% elastin). This is a ratio similar to that found in native arterial wall tissue. An example of the results of the collagen-based fibrous mat production can be seen in Figure 6 (magnification 1800X). The mats produced were approximately 100 microns thick.

Food Casing Applications

A matrix of electroprocessed collagen may be used as a food casing product. In one example, a mandrel having a specific shape (typically in the case of a sausage – a cylindrical shape) is used as the target for the streamed solution containing collagen. The thickness and strength of the matrix can be varied depending on the requirements of the food product to be packed within the matrix. Specifically desirable variations in this process may include the incorporation in the solution of various flavor or food preservative ingredients that will be incorporated into the food casing matrix. Alternatively, flavoring or preservatives may be added to the matrix after electroprocessing. The color of the matrix can also be varied. Aside from simple cylindrical shapes, more complex shapes can be seamlessly made. Trendy food products having unusual shapes are possible.

Manufactured Leather

Since leather is comprised essentially of collagen, the electroprocessing invention may be used to manufacture a leather product. Complex, seamless leather forms can be fabricated. This fabrication process would utilize the natural polymer collagen in fibrillar form to produce natural leather like products. Electrospinning leather in this fashion will provide a means to make fiber blends to produce novel fabric combinations. Novel fabric combinations could be manufactured to exhibit unique physical properties such as increased elasticity, water resistance/water proofness, increased strength, durability, and selected incorporation of resilient materials for padding and gripping. Additionally, the thickness of the leather fabric can be selected and controlled. This fabrication process further minimizes waste during production of the electrospun leather as well as finding a use for waste natural leather created as a by product of

working with natural leather products. Also, utilizing natural products is more environmentally safe.

Still further, the product and process provides a mechanism to mend natural leather or the electrospun leather described herein. The invention therefore minimizes waste resulting from imperfection or tears in natural leather. Additionally, electrospun leather could be combined with natural leather to produce hybrids that would minimize waste by capitalizing on the utilization of scrap materials typically discarded in current production methods.

Seamless materials are more waterproof and less likely to fail than standard seamed leather products. Also, it is possible to produce complex seamless three-dimensional shapes with electroprocessing that precisely fit complex shapes. Another advantage is that all of the leather would be of premium quality. The invention eliminates the need to discard a rawhide because of tears or other imperfections, because the invention fabricates the electrospun leather from the basic collagen fibers that make up leather. The quality of the manufactured leather would be absolutely uniform and dependent upon the selection and choices in the manufacturing process, not the limitations in the raw materials.

Collagen from rawhide, or any other source can be isolated and prepared for electroprocessing. For example, hide can be cut into small pieces, frozen and fragmented into small pieces, lyophilized and used as a crude mixture of raw material for electroprocessing. Other isolation procedures are also possible, i.e., acid hydrolysis or the isolation of collagen to form a gel dispersion. These procedures can be tailored to isolate collagen in a realtively pure form or a crude form (i.e., still mixed with the components elements that make up the hide in its raw state). Acid extracts of collagen may be dialized against other solvents for example water, to prepare the collagen for processing.

The collagen or crude extract can then be suspended in 1,1,1,3,3,3-hexafluoro-2-propanol or another appropriate solvent or suspension. The solution (or suspension) is then placed into a syringe or other source, charged to high voltage and directed at a grounded target. Streams of solvent containing the suspended collagen are directed at the target. As the stream bridges the gap between the source and ground, the collagen undergoes polymerization to form filaments.

As with any electrospinning process, the filament diameter and orientation can be regulated to a high degree by the reaction conditions. Adding other specific materials into the collagen can further modify material properties. For example, adding a natural material like elastin or a synthetic material like rubber can be expected to produce leather with novel elastic properties. Material properties can also be modulated by adding additional materials during the electrospinning process from additional sources (i.e. other syringes). The advantage of this strategy is that filaments of dissimilar properties can be mixed at the molecular level during fabrication, i.e. filaments of separate and distinct properties can be intermingled at the individual filament level. Spinning specific materials in sequence with one another can produce layers of materials. Also, by mixing different types of collagen or collagen that has been manipulated other ways (e.g. added or removed carbohydrates or peptides) in the solution or filament form, different textures or material properties can be achieved. Also, by forming a gradient in the collagen sources, the composition of the final product can be controlled. A collagen gradient would allow the materials to take on multiple mechanical properties within the same panel of fabric to allow the fabric to accomplish complex functions. The gradient may include variable concentrations of collagen and/or variable types of collagen or other polymer or additive. For example, a high concentration of collagen could be used to produce filaments of high mechanical strength. A gradient towards a low concentration of collagen in the source solution would

produce less filamentation and more globular material that could provide a soft surface, a gripping surface, or padding.

After electrospinning, further processing can be performed to produce varying colors, textures, scents, and resiliency (i.e. tanning). Cross linking agents (for example gluteraldehyde, UV light or other conventional tanning materials) can be applied to the product at various stages to adjust material properties. Also, there is nothing to prohibit using the final product in more traditional ways, e.g., producing sheets of the manufactured leather fabric to make products with seams.

Example 4

The collagen used was Type I (calf skin, Sigma Chemical Co.). The collagen was suspended in 1,1,1,3,3,3 -hexfluoro-2-propanol (HFP) at a concentration of 0.1181 grams in 3 ml HFP. Once in solution or suspension (solution in milky color), the solution was loaded into a 1 ml syringe plunger. A 15-gauge luer stub adapted was then placed on the syringe to act as the electrospinning nozzle and charging point for the contained collagen solution. The filled syringe was placed in the KD Scientific's syringe pump set to dispense the solution at a rate of 18 ml/hr utilizing a Becton Dickinson 1.0-ml syringe plunger. The positive lead from the high voltage supply was attached to the luer stub adapter metal portion. The syringe pump was turned on and the high voltage supply turned on and set at 20 kV. The grounded target was a 303 stainless steel mandrel (0.6 cm W x 0.05 cm H x 4 cm L) placed approximately 6 inches from the tip of the adapter. The mandrel was rotated at approximately 500 rpm during the spinning process. In the experiment, 1 ml of the collagen solution was electrospun to form a nice, white mat on the grounded mandrel. After electrospinning, the collagen mat was removed from the mandrel and

processed for scanning electron microscopy evaluation. The results of this fibrous mat production can be seen in Figures 7-9. (Magnification 800X, 8000X and 850X respectively). The mat produced was approximately 200 microns thick.

For the production of leather, the collagen mat sample was placed in 2% glutaraldehyde solution (0.1 M sodium cacodylate) for three days (over the weekend). The sample was then placed in 1% osmium tetroxide for 1 to 1.5 hours. The sample was then dehydrated with increasing ethyl alcohol solutions (50-100%). The samples were then sputter coated for viewing on the scanning electron microscope. Results of the fixed sample are shown in Figures 10-12. (Magnification 800X, 8000X and 2700X respectively). The figures show a highly cross-linked collagenous mat that is a reproduction of leather.

Footwear and Clothing Applications

The use of electroprocessing to manufacture footwear and clothing products is examined in more detail in provisional application entitled Electroprocessing Polymers To Form Footwear And Clothing, Serial No. ___________ filed on October 18, 2000 which is incorporated herein by reference as is set forth in its entirety. In short, a substrate having a desired shape, whether it be a shoe, sock, shirt, or any article of clothing is the target in the electroprocessing procedure. In this way, a custom shoe or custom piece of clothing is made that will exactly and seamlessly fit the shape of the madrel – ideally the shape of a person's foot, torso, etc. The thickness and attributes of the resulting matrix may be varied depending on the type of clothing that is desired. Also, different polymers may be incorporated into the solution to obtain the end result of a specifically desired piece of clothing or footwear.

While the invention has been described with reference to specific embodiments thereof, it will understood that numerous variations, modifications and additional embodiments are possible, and accordingly, all such variations, modifications, and embodiments are to be regarded as being within the spirit and scope of the invention.